WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 5, 207-217. Review Article ISSN 2277– 7105

# FUSOBACTERIUM NUCLEATUM- AN EMINENT PERIODONTAL PATHOGEN AND ITS ROLE IN PERIODONTITIS

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Article Received on
23 Jan. 2023,

Revised on 13 Feb. 2023,
Accepted on 03 March 2023

DOI: 10.20959/wjpr20235-27407

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## ABSTRACT

Fusobacterium nucleatum is a gram negative, cigar shaped bacillus with pointed ends, and found in both health and disease. It comes under orange complex of periodontal pathogens. The prevalence of Fusobacterium nucleatum is high in health and disease and its levels are increased in saliva and serum in patients with periodontitis. As the levels of F. nucleatum increases so does the severity of periodontal disease with inflammation and probing depth. Detection of F. nucleatum requires a method that is highly specific and sensitive. These methods include bacterial culture - based cultivation, microscopy, DNA probe hybridization, fluorescent oligonucleotide probe hybridization and gene amplification through PCR. The present article aims and describes the co-aggregating bacteria F. nucleatum and its role in periodontitis.

**KEYWORDS:-** Fusobacterium- Periodontitis- Co-aggregation bacteria- Culture methods.

## INTRODUCTION

Bacteria are causative agents of periodontal diseases. Interactions between oral bacteria and gingival epithelial cells are essential aspects of periodontal infections. Historically, Gram-negative anaerobic bacteria have been implicated as etiologic agents of periodontal diseases. Depending on the severity, the disease can be broadly classified into two stages: gingivitis,

characterized by tissue inflammation, and periodontitis, associated with attachment loss, alveolar bone resorption, and tooth loss. Among more than 300 species identified in the oral cavity, a relatively small group of gram-negative organisms, including *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Campylobacter* spp., *Capnocytophoga* spp., *Eikenella corrodens*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Prevotella intermedia*, along with oral spirochetes, are the bacteria most frequently isolated from infected periodontal pockets and are thus recognized as potential periodontal pathogens. Among the gram-negative bacteria implicated in periodontal disease *Fusobacterium nucleatum* is one of the most interesting. It is the common pathogen that significantly overgrows in periodontitis. It is one of the common microorganisms involved in halitosis and localized aggressive periodontitis.[1,2]

**Microbial characteristics**

Fusobacterium nucleatum is an anaerobic Gram-negative non-spore forming bacterium, and the type species for the genus Fuosbacterium. The cells of F. nucleatum are spindle-shaped or fusiform rods of variable length with pointed ends. Recent studies have shown that the organism obtains energy via the fermentation of carbohydrates and specific amino acids, producing butyrate as a major metabolic end-product. F. nucleatum is frequently associated with periodontal diseases, as it is a common human dental plaque isolate and based on its ability to adhere to a wide range of other plaque microorganisms it is proposed to play a crucial role in plaque development. It is one of the microorganisms detected through DNA probe.[3]

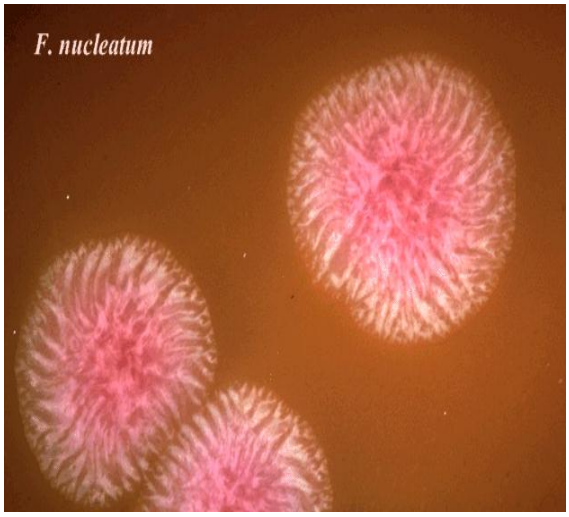


**Fig. 1: F. nucleatum – (Ultra-structure) colony morphology of fusobacterium nucleatum forming purple colonies courtesy: carranzas clinical periodontology, 10th edition.**

**Sub types**

Its sub types are:

**I.** ***Fusobacterium nucleatum*** Knorr 1922, species.[4] (Type species of the genus). — Type strain: strain ATCC 25586 = CCUG 32989 = CCUG 33059 = CIP 101130 = JCM 8532 = LMG 13131. — Synonyms: "*Bacillus fusiformis*" Veillon and Zuber 1898, "*Corynebacterium fusiforme*" (Veillon and Zuber 1898) Lehmann and Neumann 1907, "*Fusiformis nucleatus*" (Knorr 1922) Bergey *et al*. 1930, "*Fusiformis fusiformis*" (Veillon and Zuber 1898) Topley and Wilson 1936, "*Sphaerophorus fusiformis*" (Veillon and Zuber 1898) Sebald 1962,.-This species has been divided into several subspecies:

**a)** ***Fusobacterium nucleatum*** **subsp.** ***animalis*** Gharbia and Shah 1992, subsp. nov. — Type strain: strain ATCC 51191 = CCUG 32879 = CIP 104879 = NCTC 12276.

**b)** ***Fusobacterium nucleatum*** **subsp.** ***fusiforme*** (*ex* Veillon and Zuber 1898) Gharbia and Shah 1992, subsp. nov., nom. rev., comb. nov. — Type strain: strain ATCC 51190 = CCUG 32880 = CIP 104878 = NCTC 11326.

**c)** ***Fusobacterium nucleatum*** **subsp.** ***nucleatum*** Knorr 1922, subsp. nov. — Type strain: strain ATCC 25586 = CCUG 32989 = CCUG 33059 = CIP 101130 = JCM 8532 = LMG 13131.[5]

**d)** ***Fusobacterium nucleatum*** **subsp.** ***polymorphum*** (*ex* Knorr 1922) Dzink *et al*. 1990, subsp. nov., nom. rev., comb. nov. — Type strain: strain ATCC 10953 = CCUG 9126 = DSM 20482 = NCTC 10562.[6]

**e)** ***Fusobacterium nucleatum*** **subsp.** ***vincentii*** (*ex* Knorr 1922) Dzink *et al*. 1990, subsp. nov., nom. rev., comb. nov. — Type strain: strain ATCC 49256 = CIP 104988.[7]

[*During the meeting of International Committee on Systematics of Prokaryotes, 29 July 2002, Paris, the members of the "Subcommittee on the taxonomy of Gram-negative anaerobic rods" were of the opinion that the subspecies of Fusobacterium nucleatum, which have been questioned periodically, are valid and have now been confirmed by sequencing of the spacer region of the 16S-23S rDNA. —(Reference: OLSEN (I.) and SHAH (H.N.):. Minutes of the meeting, 29 July 2002, Paris, France. Int. J. Syst. Evol. Microbiol., 2003, **53**, 923-924*)].[8]

II. ***Fusobacterium perfoetens*** (Tissier 1905) Moore and Holdeman 1973, *species*. — Type strain: strain ATCC 29250.

III. ***Fusobacterium periodonticum*** Slots *et al*. 1984, sp. nov. — Type strain: strain EK1-15 = ATCC 33693 = CCUG 14345

IV. ***Fusobacterium plautii*** corrig. Séguin 1928, *species*. — Type strain: strain Prévot S1 = ATCC 29863 = CCUG 28093 = DSM 4000 = VPI 0310

V. ***Fusobacterium polysaccharolyticum*** van Gylswyk 1981, sp. nov. — Type strain: strain B = ATCC 33142.

**1. Drug susceptibility**

Sensitive to penicillin, clindamycin, chloramphenicol.

**2. Drug resistance**

Erythromycin and macrolides.

**3. Susceptibility to disinfectants**

Susceptible to disinfectants - 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, iodine, phenolics, formaldehyde.

**4. Physical inactivation**

Sensitive to moist heat (121° C for at least 15 min) and dry heat (160-170° C for at least 1 hour)

**5. Survival outside host**

Manure - 292 days; culture exposed to air -7 days; soil - 56 days.[9,10]

**6. Epidemiology**

Worldwide; F. nucleatum is the most common Fusobacterium species found in clinical infections, however, F. necrophorum is most virulent species.

**7. Pathogenicity**

Indigenous flora on all mucosal surfaces, normal inhabitants of human cavities (mouth, upper respiratory tract, gastro intestinal tract and urogenital tract); can cause purulent or gangrenous infections; infections involve all regions of body - respiratory, urogenital and gastrointestinal tracts, abscesses, septicemia, pleurisy, necrotizing gingivitis; usually occurring when mucosal damage related to surgery, trauma or disease occurs.

**F. nucleatum in biofilm colonization**

The bacterial species of biofilms are placed in either of two general categories, early colonizers or late colonizers. This placement is based on the species of bacteria identified in dental plaque during temporal sampling after oral hygiene procedures. Fusobacterium nucleatum, however, is unusual and is intentionally placed at the border between early and late colonizers for the following reasons. First, F. nucleatum is the most numerous gram-negative species in healthy sites, and its numbers increase markedly in periodontally diseased sites. It is always present whenever Treponema denticola and Porphyromonas gingivalis are also present, suggesting that its presence predates that of the other two species and may be required for their colonization.[11] Second, F. nucleatum coaggregates with all the early colonizers and the late colonizers. The bacteria representing early colonizers coaggregate with only a specific set of other early colonizers but not with all of them and generally not with any of the late colonizers. Although all the late colonizers coaggregate with F. nucleatum, they generally do not coaggregate with each other. Thus, F. nucleatum acts as a bridge between early and late colonizers, which may partially explain why fusobacteria are so numerous in samples from both healthy and diseased sites.

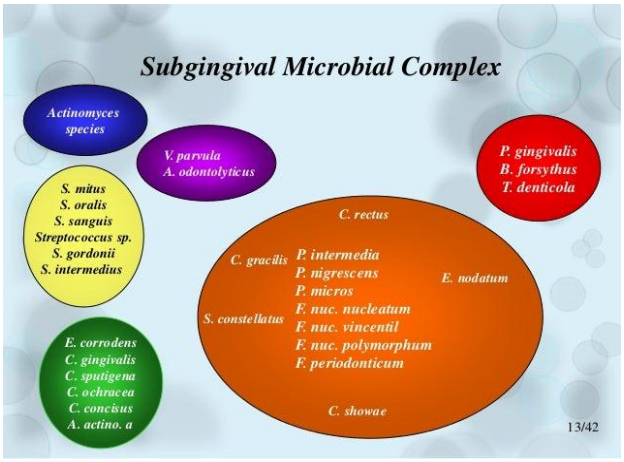


**Fig. 2: Bacterial complexes- schematic representation showing microbial complexes in subgingival plaque courtesy- textbook of periodontics, 2nd edition shalu bathla.**

**F. nucleatum in biofilm coaggregation**

Coaggregation bridges are mechanisms of cooperation because they bring together two species that are not coaggregation partners. Such bridges may be critical for temporary retention of bacteria on a nascent surface and may facilitate eventual bacterial colonization of the biofilm. The bridges are distinct from competition, which occurs when multiple species compete for binding to the same receptor. Competitive and cooperative mechanisms may be

central to successful mixed-species colonization as well as the proper succession of genera known to occur on teeth in both health and disease. The lactose-inhibitable coaggregations between F. nucleatum and its partners appear to be mediated by the same galactose-binding adhesin that mediates attachment of F. nucleatum to mammalian cells, including human buccal epithelial cells and gingival and periodontal ligament fibroblasts. A recent study reported galactose-inhibitable binding and invasion of human gingival epithelial cells by F. nucleatum.[12] These results attribute great potential significance to galactose-sensitive adhesins for initiating communication between early and late colonizers as well as with their host.

Finally, in addition to interactions with oral bacteria and host cells, F. nucleatum interacts with and binds host-derived molecules, such as plasminogen. F. nucleatum is generally nonproteolytic, but organisms that coexist with it, such as P. gingivalis, are highly proteolytic and can activate fusobacterium-bound plasminogen to form fusobacterium-bound plasmin, a plasma serine protease. Acquisition of proteolytic ability on its cell surface confers on the fusobacteria a new metabolic property, the ability to process potential peptide signals in the community. These peptides may be used as nutrients by fusobacteria or by other biofilm residents.[13] F. nucleatum also induces expression of ß-defensin 2, a small cationic peptide produced by mucosal epithelial cells. P. gingivalis does not elicit ß-defensin 2 production, suggesting a distinction between these two important oral bacteria and their role in stimulating innate immune responses.[14,15] Although F. nucleatum is often considered a periodontal pathogen, it may instead contribute to maintaining homeostasis and improving host defense against true pathogens.

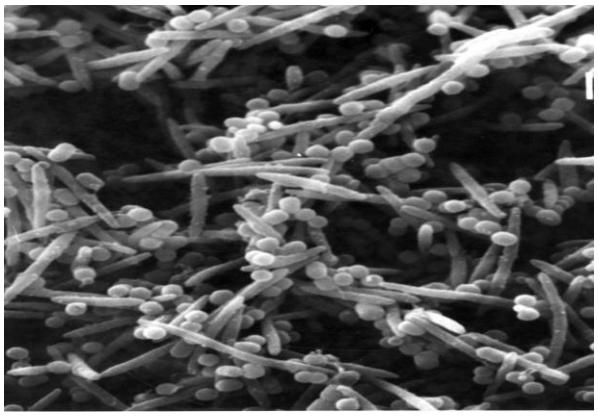

**Fig. 3: Co-aggregation of f. nucleatum courtesy: human genome sequencing center (Baylor college of medicine).**

**Role of f. nucleatum in periodontitis**

F. nucleatum is a filamentous human pathogen strongly associated with periodontal diseases, as well as infections and abscesses in other parts of the body. The pathogenic potential of Fusobacterium nucleatum and its significance in the development of periodontal diseases, as well as in infections in other organs, have gained new interest for several reasons.

First, this bacterium has the potential to be pathogenic because of its number and frequency in periodontal lesions, its production of tissue irritants, its synergism with other bacteria in mixed infections, and its ability to form aggregates with other suspected pathogens in periodontal disease and thus act as a bridge between early and late colonizers on the tooth surface. This organism can induce apoptotic cell death in mononuclear and PMNs.[16] It can trigger the release of elastase, cytokines and oxygen radicals from leucocytes.[17,18]

Second, of the microbial species that are statistically associated with periodontal disease, F. nucleatum is the most common in clinical infections of other body sites. Third, during the past few years, new techniques have made it possible to obtain more information about F. nucleatum on the genetic level, thereby also gaining better knowledge of the structure and functions of the outer membrane proteins (OMPs).[19,20] OMPs are of great interest due to their role in coaggregation, cell nutrition, and antibiotic susceptibility. Investigation of virulence factors should improve our understanding of the role of *F. nucleatum* in periodontal infections.

Fourthly, *F. nucleatum* adheres to and invades the human gingival epithelial cells and that this was accompanied by high levels of IL-8 secretion from the epithelial cells. Invasion appeared to occur via a "zipping" mechanism and required the involvement of actins, microtubules, signal transduction, protein synthesis, and energy metabolism of the epithelial cell, as well as protein synthesis by *F. nucleatum.*[21] Interactions between bacteria and their surrounding epithelium are critical factors in bacterial infections Adherence to epithelial cells is important for colonization. It can directly invade the host cell. Invasion allows the bacteria not only to evade the host immune surveillance but also to spread into deeper tissues. Histological studies of periodontal infections also indicated penetration of deeper tissues by cocci, rods, and fuso-spirochetal forms of bacteria in advanced periodontitis.

So far, analysis of tissue attachment and invasion by oral bacteria has been focused on *A. actinomycetemcomitans* and *P. gingivalis*. Both organisms invade by a "ruffling"

mechanism; that is, they cause dramatic ruffling of host cell membranes at the site of entry, resulting in bacteria internalized in the form of spacious vacuoles. This ruffling mechanism is one of the two major penetration mechanisms used by invasive bacteria. The other major entry mechanism, termed zipping, in which the invading bacteria remain in close contact with the host membrane during penetration, has not been reported for any oral bacteria till now, except for F.nucleatum.

**Pathogenesis**

Three biological activities are often associated with virulence:

1) Bacterial attachment,
2) Invasion, and
3) Induction of IL-8 production.

Adherence is a common characteristic shared by many pathogens since it is a crucial step for establishing an infection. F.nucleatum plays an important role in adherence of other organisms to the biofilm by facilitating co-aggregation of late colonizers with early colonizers. F.nucleatum also has the potential to interact with the epithelium to establish colonization and is reported to be highly invasive, with its activity being comparable to that of P. gingivalis. Several adherence modes and putative adhesins have been proposed for F. nucleatum and it is possible that one or more adhesins are involved in the various adherence and invasion processes. Electron microscopy has revealed clear evidence of invasion of fibroblast cells by F. nucleatum. Due to the distinctly large size of this organism, different stages of penetration are easily visualized under SEM. It appears that F. nucleatum invades both cell types via a zipping mechanism, which is the only reported incidence of this mechanism for an oral bacterium. Upon internalization, the organism apparently resides in vacuoles. Furthermore, metabolic inhibition assays showed that invasion required the participation of host actin, microtubules, signal transduction, and energy metabolism.[22]

Invasion required protein synthesis by F. nucleatum as well as other nutrient productions. The adherence of F. nucleatum to fibroblasts and epithelial cells is reported to involve galactose-binding lectin(s), since the attachment was greatly inhibited by galactose-containing sugars. This property is currently being utilized to identify adhesins of this organism. It is possible that a non-lectin-like adhesin(s) may also be involved in the interactions between F. nucleatum and the host cells. Besides bacterial penetration into deeper tissues, one other important characteristic of periodontal infection is inflammation, a result of neutrophil

infiltration into infected sites. Continuous production of proinflammatory cytokines, such as IL-8, appears to be important for the progression of periodontal infections and tissue destruction. The initiation and progression of periodontal infections may be affected by the dynamic competition between suppression and stimulation of immune mediators by various periodontal bacteria. Diaz etal found that F. nucleatum is likely to be a strong stimulator of IL-8 production throughout the course of infection. This conclusion is consistent with clinical studies demonstrating that F. nucleatum is highly prevalent during the early stages of inflammation associated with gingivitis.

## CONCLUSION

Periodontal diseases result from complex actions of a group of periodontal bacteria, mostly gram-negative anaerobes. However, so far only a few, such as A. actinomycetemcomitans and P. gingivalis, have been characterized as bonafide periodontal pathogens. Other gram-negative organisms, although often linked to various forms of periodontal diseases, are considered only putative pathogens, largely due to our limited understanding of their virulence potential. With its length of 10 times that of Escherichia coli, F. nucleatum is by far the largest bacteria involved in the cellular processes of invasion of Gingival epithelial and connective tissue components. In addition, F. nucleatum is an important contributor to periodontal disease by serving as a co-aggregating mediator of plaque biofilm colonization by other virulent anaerobes. F.nucleatum not only highly invasive but also adheres to crevicular/junctional epithelial cells; and is a potent stimulator of IL-8 expression by these cells. Hence F.nucleatum is now recognized to be an important Periodonto-pathogen and its elimination by antibiotic administration and surgical or non-surgical periodontal therapy is of primary importance in managing plaque associated chronic periodontal infections.

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